

What Is Claimed Is:

1. A method for enhancing the uptake of a molecule into a metabolically active whole cell, comprising incubating said metabolically active whole cell with said molecule in the presence of an agent that causes increased uptake of molecules into metabolically active cells, said agent being present at a concentration sufficient to enhance the uptake of said substrate or analyte compound into said metabolically active whole cell.
- 5
2. The method of claim 1, wherein said agent that causes increased uptake of molecules into metabolically active cells is selected from the group consisting of glycerol, dimethyl sulfoxide (DMSO), trehalose, glutamate, betaine, ethylene glycol, threitol, ribose, and trimethylamine N-oxide.
- 10
3. The method of claim 1, wherein said molecule permits the staining, imaging or visualization of said cell or of structures, regions or molecules within said cell.
- 15
4. The method of claim 1, wherein said molecule is selected from the group consisting of a lectin, a nucleic acid, a paramagnetic moiety, an enzyme substrate or an analyte.
-
5. A method for assaying for the presence or activity of an enzyme in a metabolically active whole cell, comprising the steps:
- (a) incubating said metabolically active whole cell with a substrate of said enzyme or an analyte compound in the presence of an agent that enhances uptake of said substrate or analyte, said agent being present at a concentration sufficient to enhance the uptake of said substrate or analyte compound;
- 20
- (b) assaying for any change in concentration of said substrate or analyte compound or of a product formed via action of said enzyme on said substrate or analyte;
- 25

wherein a change in said concentration is indicative of the presence or activity of said enzyme in said metabolically active whole cell.

6. The method of claim 5, wherein said enzyme is selected from the group consisting of a 5' nucleotidase, acetylcholinesterase, an acid phosphatase, an acidic esterase, an acidic esterase I, an acidic esterase II, an acidic non-specific esterase, an adenosine deaminase, an adenosine monophosphate deaminase, an alkaline phosphatase, an aminopeptidase A, an aminopeptidase B, an aminopeptidase M, an Aminopeptidase N, an angiotensin converting enzyme, a caspase, a cathepsin B, a cathepsin B1, a cathepsin C, a cathepsin D, a cathepsin H, a cathepsin L, a cholinesterase, a cholinesterase, a chymotrypsin, a collagenase, a cytosine deaminase, a DPP I, a DPP II, a DPP IV, an elastase, an endopeptidase I, an endopeptidase II, an ester proteinase, a galactopyranosidase, a glucuronidase, a glutathione, a glycopyranosidase, a guanine deaminase, an HIV Protease, a lipase, a membrane associated endopeptidase I, a membrane associated endopeptidase II, a neutral endopeptidase, a neutral esterase, a neutral esterase I, a neutral esterase II, a neutral non-specific esterase, a nucleosidase, a pancreatic, a phospholipase A, a phospholipase C, a phospholipase D, a plasmin, a serine phosphatase, a tartrate resistant phosphatase, a tartrate resistant phosphatase, a threonine phosphatase, a thymidine deaminase, a tripeptidyl peptidase, a trypsin, a tyrosine phosphatase, a urokinase, a v-thrombin, and a γ -GT.
- 10
- 15
7. The method of claim 6, wherein said enzyme is a caspase.
- 20
- 25
8. The method of claim 7, wherein said caspase is caspase 1, caspase 3, caspase 6, caspase 8 or caspase 9.
9. The method of claim 5, wherein said substrate or analyte compound of said enzyme and said agent that enhances uptake are mixed during said incubation.

- JULY 10 1997
FBI LABORATORY
- Sub A*
10. The method of claim 5, wherein said substrate or analyte compound of said enzyme and said agent that enhances uptake are not mixed during said incubation.
 11. The method of claim 5, wherein multiple enzymes are assayed simultaneously assayed.
 12. The method of claim 5, wherein multiple enzymes are sequentially assayed.
 13. The method of claim 5, wherein said substrate or analyte compound comprises comprising an indicator group and one or more leaving groups, each of said leaving groups being selected for cleavage by said enzyme, said indicator group being in a first state when bonded to a leaving group, and being in a second state when said leaving group is cleaved from said indicator group by said enzyme; and wherein said step (b) comprises sensing whether said second state of said indicator group is produced; wherein the production of said second state of said indicator group is indicative of the presence or activity of said enzyme in said metabolically active whole cell.
 14. The method of claim 13, wherein said indicator group is a fluorescent, colorimetric, bioluminescent or chemiluminescent indicator group.
 15. The method of claim 5 or claim 13, wherein said uptake-enhancing agent is selected from the group consisting of glycerol, dimethyl sulfoxide (DMSO), trehalose, glutamate, betaine, ethylene glycol, threitol, ribose, and trimethylamine N-oxide.
 20. The method of claim 15, wherein said uptake-enhancing agent is glycerol.
 25. The method of claim 16, wherein said glycerol concentration is between about 5% and about 60% (v/v).
 18. The method of claim 17, wherein said glycerol concentration is between about 20% and about 60% (v/v).

- 15
Sur a
19. The method of claim 18, wherein said glycerol concentration is between about 25% and about 40% (v/v).
 20. The method of claim 15, wherein said uptake-enhancing agent is dimethyl sulfoxide (DMSO).
 - 5 21. The method of claim 20, wherein said dimethyl sulfoxide concentration is between about 5% and about 60% (v/v).
 22. The method of claim 21, wherein said dimethyl sulfoxide concentration is between about 20% and about 60% (v/v).
 23. The method of claim 15, wherein said uptake-enhancing agent is glutamate.
 - 10 24. The method of claim 23, wherein said glutamate concentration is between about 0.25 M and about 2.0 M.
 25. The method of claim 24, wherein said glutamate concentration is between about 1 M and about 2 M.
 26. The method of claim 15, wherein said uptake-enhancing agent is betaine.
 - 15 27. The method of claim 26, wherein said betaine concentration is about 0.3 M or greater.
 28. The method of claim 15, wherein said uptake-enhancing agent is trehalose.
 29. The method of claim 28, wherein said trehalose concentration is between about 0.1 M and about 1.5 M.
 - 20 30. The method of claim 15, wherein said uptake-enhancing agent is ethylene glycol.
 31. The method of claim 30, wherein said ethylene glycol concentration is between about 2 M and about 7 M.
 32. The method of claim 15, wherein said uptake-enhancing agent is threitol.

33. The method of claim 32, wherein said threitol concentration is between about 1 M and about 5 M.
34. The method of claim 15, wherein said uptake-enhancing agent is ribose.
35. The method of claim 34, wherein said ribose concentration is between about 0.4 M and about 4 M.
36. The method of claim 15, wherein said uptake-enhancing agent is triethylamine N-oxide.
37. The method of claim 36, wherein said triethylamine N-oxide concentration is between about 0.4 M and about 4 M.
- 10 38. The method of claim 13, wherein said enzyme is selected from the group consisting of a 5' nucleotidase, acetylcholinesterase, an acid phosphatase, an acidic esterase, an acidic esterase I, an acidic esterase II, an acidic non-specific esterase, an adenosine deaminase, an adenosine monophosphate deaminase, an alkaline phosphatase, an aminopeptidase A, an aminopeptidase B, an aminopeptidase M, an Aminopeptidase N, an angiotensin converting enzyme, a caspase, a cathepsin B, a cathepsin B1, a cathepsin C, a cathepsin D, a cathepsin H, a cathepsin L, a cholinesterase, a cholinesterase, a chymotrypsin, a collagenase, a cytosine deaminase, a DPP I, a DPP II, a DPP IV, an elastase, an endopeptidase I, an endopeptidase II, an ester proteinase, a galactopyranosidase, a glucuronidase, a glutathione, a glycopyranosidase, a guanine deaminase, an HIV Protease, a lipase, a membrane associated endopeptidase I, a membrane associated endopeptidase II, a neutral endopeptidase, a neutral esterase, a neutral esterase I, a neutral esterase II, a neutral non-specific esterase, a nucleosidase, a pancreatin, a phospholipase A, a phospholipase C, a phospholipase D, a plasmin, a serine phosphatase, a tartrate resistant phosphatase, a tartrate resistant phosphatase, a threonine phosphatase, a thymidine deaminase, a tripeptidyl peptidase, a trypsin, a tyrosine phosphatase, a urokinase, a v-thrombin, and a γ-GT.

- TO SIGHT: 6498/6600
Jul 11
39. The method of claim 38, wherein said enzyme is a caspase.
40. The method of claim 39, wherein said caspase is caspase 1, caspase 3, caspase 6, caspase 8, or caspase 9.
41. The method of claim 13, wherein multiple enzymes are simultaneously assayed.
- 5 42. The method of claim 13, wherein multiple enzymes are sequentially assayed.
43. The method of claim 13, wherein said step (b) includes measuring an intensity of said second state against time.
- 10 44. The method of claim 13, wherein said step (b) includes measuring a magnitude of said second state at a point of time.
45. The method of claim 13, wherein said substrate or analyte compound comprises more than one leaving group, and wherein each of said substrate's leaving groups is cleaved sequentially by said enzyme.
- 15 46. The method of claim 13, wherein said indicator group is selected from the group consisting of rhodamine 110, rhodol, fluorescein, coumarin, and derivatives thereof.
47. The method of claim 46, wherein said derivatives of rhodamine 110, rhodol, fluorescein and coumarin are selected from the group consisting of 4'(5')thiofluorescein, 4'(5')-aminofluorescein, 4'(5')-carboxyfluorescein, 4'(5')-chlorofluorescein, 4'(5')-methylfluorescein, 4'(5')-sulfofluorescein, 4'(5')-aminorhodol, 4'(5')-carboxyrhodol, 4'(5')-chlororhodol, 4'(5')-methylrhodol, 4'(5')-sulforhodol; 4'(5')-aminorhodamine 110, 4'(5')-sulforhodamine 110, 4'(5')thiorhodamine 110, 7-aminocoumarin, and sulfonated coumarin.
- 20
- 25

48. The method of claim 13, wherein said assay detects the presence or absence of an abnormality in the activity of said enzyme by comparing the production of said second state of said indicator group by said test cell to the production of said second state of said indicator group by a reference normal cell.
- 5
49. The method of claim 48, wherein said abnormality is a morphological or disease state.
50. The method of claim 49, wherein said morphological state is an apoptotic state.
- 10 51. The method of claim 49, wherein said disease state is a tumorigenic state.
52. The method of claim 13, wherein said substrate or analyte compound contains a blocking group.
53. The method of claim 52, wherein said blocking group is a Cbz blocking group.
- 15 54. The method of claim 13, wherein said substrate or analyte compound of said enzyme and said agent that enhances said uptake are mixed during said incubation.
55. The method of claim 13, wherein said substrate or analyte compound of said enzyme and said agent that enhances said uptake are not mixed during said incubation.
- 20
56. A reagent for assaying the activity of an enzyme, said reagent comprising a substrate of said enzyme or an analyte compound and an agent that enhances said uptake, said agent being present at a concentration sufficient to enhance the uptake of said substrate or analyte compound in a metabolically active cell.
- 25

- 100-100-100-100-100
- Sub A1*
57. The reagent of claim 56, wherein said enzyme is selected from the group consisting of a 5' nucleotidase, acetylcholinesterase, an acid phosphatase, an acidic esterase, an acidic esterase I, an acidic esterase II, an acidic non-specific esterase, an adenosine deaminase, an adenosine monophosphate deaminase, an alkaline phosphatase, an aminopeptidase A, an aminopeptidase B, an aminopeptidase M, an Aminopeptidase N, an angiotensin converting enzyme, a caspase, a cathepsin B, a cathepsin B1, a cathepsin C, a cathepsin D, a cathepsin H, a cathepsin L, a cholinesterase, a cholinesterase, a chymotrypsin, a collagenase, a cytosine deaminase, a DPP I, a DPP II, a DPR IV, an elastase, an endopeptidase I, an endopeptidase II, an ester proteinase, a galactopyranosidase, a glucuronidase, a glutathione, a glycopyranosidase, a guanine deaminase, an HIV Protease, a lipase, a membrane associated endopeptidase I, a membrane associated endopeptidase II, a neutral endopeptidase, a neutral esterase, a neutral esterase I, a neutral esterase II, a neutral non-specific esterase, a nucleosidase, a pancreatin, a phospholipase A, a phospholipase C, a phospholipase D, a plasmin, a serine phosphatase, a tartrate resistant phosphatase, a tartrate resistant phosphatase, a threonine phosphatase, a thymidine deaminase, a tripeptidyl peptidase, a trypsin, a tyrosine phosphatase, a urokinase, a v-thrombin, and a γ -GT.
58. The reagent of claim 57, wherein said enzyme is a caspase.
59. The reagent of claim 58, wherein said caspase is caspase 1, caspase 3, caspase 6, caspase 8 or caspase 9.
60. The reagent of claim 56, wherein said substrate or analyte compound comprises comprising an indicator group and one or more leaving groups, each of said leaving groups being selected for cleavage by said enzyme, said indicator group being in a first state when bonded to a leaving group, and being in a second state when said leaving group is cleaved from said indicator group by said enzyme; and wherein said step (b) comprises sensing whether said second state of said indicator group is produced;

wherein the production of said second state of said indicator group is indicative of the presence or activity of said enzyme in said metabolically active whole cell.

61. The reagent of claim 60, wherein said indicator group is a fluorescent, colorimetric, bioluminescent or chemiluminescent indicator group.
- 5 62. The reagent of claim 61, wherein said indicator group is a fluorescent or chemiluminescent indicator group.
63. The reagent of claim 56 or claim 61, wherein said uptake-enhancing agent is selected from the group consisting of glycerol, dimethyl sulfoxide (DMSO), trehalose, glutamate, betaine, ethylene glycol, threitol, ribose, and trimethylamine N-oxide.
- 10 64. The reagent of claim 63, wherein said uptake-enhancing agent is glycerol.
65. The reagent of claim 64, wherein said glycerol concentration is between about 5% and about 60% (v/v).
- 15 66. The reagent of claim 65, wherein said glycerol concentration is between about 20% and about 60% (v/v).
67. The reagent of claim 66, wherein said glycerol concentration is between about 25% and about 40% (v/v).
- 20 68. The reagent of claim 63, wherein said uptake-enhancing agent is dimethyl sulfoxide (DMSO).
69. The reagent of claim 68, wherein said dimethyl sulfoxide concentration is between about 5% and about 60% (v/v).
70. The reagent of claim 69, wherein said dimethyl sulfoxide concentration is between about 20% and about 60% (v/v).
- 25 71. The reagent of claim 63, wherein said uptake-enhancing agent is glutamate.

- TOSPTOT:864882660
SJL a1
- 72. The reagent of claim 71, wherein said glutamate concentration is between about 0.25 M and about 2.0 M.
 - 73. The reagent of claim 72, wherein said glutamate concentration is between about 1 M and about 2 M.
 - 5 74. The reagent of claim 63, wherein said uptake-enhancing agent is betaine.
 - 75. The reagent of claim 74, wherein said betaine concentration is about 0.3 M or greater.
 - 76. The reagent of claim 63, wherein said uptake-enhancing agent is trehalose.
 - 10 77. The reagent of claim 76, wherein said trehalose concentration is between about 0.1 M and about 1.5 M.
 - 78. The reagent of claim 63, wherein said uptake-enhancing agent is ethylene glycol.
 - 79. The reagent of claim 78, wherein said ethylene glycol concentration is between about 2 M and about 7 M.
 - 15 80. The reagent of claim 63, wherein said uptake-enhancing agent is threitol.
 - 81. The reagent of claim 80, wherein said threitol concentration is between about 1 M and about 5 M.
 - 82. The reagent of claim 63, wherein said uptake-enhancing agent is ribose.
 - 83. The reagent of claim 82, wherein said ribose concentration is between about 0.4 M and about 4 M.
 - 20 84. The reagent of claim 63, wherein said uptake-enhancing agent is triethylamine N-oxide.
 - 85. The reagent of claim 84, wherein said triethylamine N-oxide concentration is between about 0.4 M and about 4 M.

- TOP SECRET//~~REF ID: A67492~~
- but at*
86. The reagent of claim 61, wherein said enzyme is selected from the group consisting of a 5' nucleotidase, acetylcholinesterase, an acid phosphatase, an acidic esterase, an acidic esterase I, an acidic esterase II, an acidic non-specific esterase, an adenosine deaminase, an adenosine monophosphate deaminase, an alkaline phosphatase, an aminopeptidase A, an aminopeptidase B, an aminopeptidase M, an Aminopeptidase N, an angiotensin converting enzyme, a caspase, a cathepsin B, a cathepsin B1, a cathepsin C, a cathepsin D, a cathepsin H, a cathepsin L, a cholinesterase, a cholinesterase, a chymotrypsin, a collagenase, a cytosine deaminase, a DPP I, a DPP II, a DPP IV, an elastase, an endopeptidase I, an endopeptidase II, an ester proteinase, a galactopyranosidase, a glucuronidase, a glutathione, a glycopyranosidase, a guanine deaminase, an HIV Protease, a lipase, a membrane associated endopeptidase I, a membrane associated endopeptidase II, a neutral endopeptidase, a neutral esterase, a neutral esterase I, a neutral esterase II, a neutral non-specific esterase, a nucleosidase, a pancreatic, a phospholipase A, a phospholipase C, a phospholipase D, a plasmin, a serine phosphatase, a tartrate resistant phosphatase, a tartrate resistant phosphatase, a threonine phosphatase, a thymidine deaminase, a tripeptidyl peptidase, a trypsin, a tyrosine phosphatase, a urokinase, a v-thrombin, and a γ -GT.
87. The reagent of claim 62, wherein said substrate or analyte compound comprises more than one leaving group, and wherein each of said substrate's leaving groups is cleaved consecutively by said enzyme to ultimately yield a free dye.
88. The reagent of claim 62, wherein said indicator group is selected from the group consisting of rhodamine 110, rhodol, fluorescein, coumarin, and derivatives thereof.
89. The reagent of claim 88, wherein said derivatives of rhodamine 110, rhodol, fluorescein and coumarin are selected from the group consisting of 4'(5')thiofluorescein, 4'(5')-aminofluorescein, 4'(5')-carboxyfluorescein,

- 4'(5')-chlorofluorescein, 4'(5')-methylfluorescein, 4'(5')-sulfofluorescein,
4'(5')-aminorhodol, 4'(5')-carboxyrhodol, 4'(5')-chlororhodol, 4'(5')-
methylrhodol, 4'(5')-sulforhodol; 4'(5')-aminorhodamine 110, 4'(5')-
sulforhodamine 110, 4'(5')thiorhodamine 110, 7-aminocoumarin, and
sulfonated coumarin.
- 5
90. The reagent of claim 61, wherein said assay detects the presence or absence
of an abnormality in the activity of said enzyme by comparing the
production of said second state of said indicator group by said test cell to
the production of said second state of said indicator group by a reference
normal cell.
- 10
91. The reagent of claim 90, wherein said abnormality is a morphological or
disease state.
92. The reagent of claim 91, wherein said morphological state is an apoptotic
state.
- 15
93. The reagent of claim 91, wherein said disease state is a tumorigenic state.
94. The reagent of claim 61, wherein said substrate or analyte compound
contains a blocking group.
95. The reagent of claim 94, wherein said blocking group is a Cbz blocking
group.